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     (FILE 'HOME' ENTERED AT 12:22:19 ON 09 DEC 2003)
     FILE 'CA' ENTERED AT 12:22:29 ON 09 DEC 2003
          E MATTIASSON B/AU
L1     562 S E3-8
L2       1 S L1 AND NILSSON H?/AU AND OLSSON B?/AU
L3       1 S L1 AND (APOENZYME OR APO(1A)ENZYME)
L4      28 S L1 AND ELECTRODE
L5       1 S L1 AND CAPACIT?(5A)(DETECT? OR SENSOR OR MONITOR)
L6   37435 S FUSION(1A)(PROTEIN OR PEPTIDE)
L7    2324 S L6 AND(GST? OR SMTA OR MERR OR MERP OR PBRR)
L8     208 S L7 AND(METAL OR ZINC OR ZN OR ZN2 OR MERCURY OR HG OR HG2 OR CADMIUM
          OR CD2 OR CD OR COPPER OR CUPRIC OR CU OR CU2 OR LEAD OR PB OR PB2)
L9 443973 S (METAL OR ZINC OR ZN OR ZN2 OR MERCURY OR HG OR HG2 OR CADMIUM OR CD2
          OR CD OR COPPER OR CUPRIC OR CU OR CU2 OR LEAD OR PB OR
          PB2)(6A)(DETECT? OR DETERMIN? OR ASSAY? OR ANALY? OR TEST? OR MEASUR?
          OR MONITOR? OR ASSES? OR SENSE# OR SENSING OR PROBE# OR PROBING OR
          ESTIMAT? OR EXAMIN?)
L10    318 S L6 AND L9
L11     52 S L7 AND(ELECTRODE OR MICROELECTRODE OR CAPACIT?)
L12     18 S L10 AND(ELECTRODE OR MICROELECTRODE OR CAPACIT?)
L13     36 S L8 AND L9
L14    125 S L2-5,L11-13
L15     78 S L14 NOT PY>1999
L16      7 S L14 NOT L15 AND PATENT/DT
L17      1 S L15 AND(SHI J? OR FRANZ B?)/AU
          E SHI J/AU
L18      1 S SHI J?/AU AND FEBS/SO AND 1992/PY
L19      1 S FRANTZ B?/AU AND 1990/PY AND BIOCHEM?/SO
L20  10525 S METAL(A)(BIND? OR RESPON?)
L21    120 S L6 AND L20
L22     64 S L21 NOT PY>1999
L23     26 S L21 NOT L22 AND PATENT/DT
L24    174 S L15-19,L22-23
     FILE 'BIOSIS' ENTERED AT 13:05:45 ON 09 DEC 2003
L25    107 S L24
     FILE 'MEDLINE' ENTERED AT 13:10:40 ON 09 DEC 2003
L26    158 S L24
     FILE 'CA' ENTERED AT 13:15:03 ON 09 DEC 2003
L27    194 S METAL(A)(BIND? OR RESPON? OR AFFINITY)AND(ELECTRODE OR
          MICROELECTRODE)
L28     18 S L27 AND(PROTEIN OR PEPTIDE OR APOENZYME OR APO(A)ENZYME)
L29     14 S (L28 NOT PY>1999)OR(L28 AND PATENT/DT)
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L30      4 S L29
     FILE 'MEDLINE' ENTERED AT 13:19:18 ON 09 DEC 2003
L31      7 S L29
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L32    263 DUP REM L24 L29 L25 L30 L26 L31 (201 DUPLICATES REMOVED)

=> d bib,ab l32 1-263

L32  ANSWER 10 OF 263  CA  COPYRIGHT 2003 ACS on STN
AN   136:396970  CA
TI   Recombinant streptavidin-metallothionein chimeric protein having biological
     recognition specificity
IN   Sano, Takeshi; Glazer, Alexander N.; Cantor, Charles R.
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PA The Regents of the University of California, USA
SO U.S., 18 pp.
PI US 6391590 B1 20020521 US 1991-780717 19911021
PRAI US 1991-780717 19911021
AB Streptavidin-metallothionein chimeric proteins with biol. recognition specificity in which the streptavidin moiety provides high affinity biotin binding and the metallothionein moiety provides a high affinity **metal binding**. The binding affinity of the streptavidin-metallothionein chimeric protein both for biotin and heavy metal ions allows specific incorporation into, conjugation with, or labeling of any biol. material contg. biotin with various heavy metal ions. Streptavidin-metallothionein chimeric protein can be used in imaging of tumor for radiotherapeutics and det. DNA sequences. 109Cd can be used to label streptavidin-metallothionein chimeric protein and then the chimeric protein contg. 109Cd is targeted to biotinylated macromols.

L32 ANSWER 19 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 133:173051 CA
TI Protein and cDNA sequences of lead-binding monoclonal antibody light chain regions and the uses thereof
IN Wylie, Dwane E.; Lopez, Osvaldo; Murray, Peter Joseph; Goebel, Peter
PA Bionebraska Inc., USA
SO U.S., 56 pp., Cont.-in-part of U.S. Ser. No. 541,373, abandoned.
PI US 6111079 A 20000829 US 1996-767128 19961204
PRAI US 1995-462798 B2 19950605
AB The present invention provides protein and cDNA sequences of a **metal binding** protein which selectively binds with a heavy metal, such as leas cation. The **metal binding** proteins which include an amino acid sequence coding for a light chain variable region of a monoclonal antibody capable of immunoreacting with a lead cation and nucleotides which include a nucleic acid sequence coding for the variable region are provided. The invention is also directed to **fusion proteins** and Fab fragments which include the light chain variable region. The invention also relates to methods for detecting, removing, adding, or neutralizing the heavy metals in biol. and inanimate systems through the use of the **metal binding** protein, heavy and light chains, **fusion proteins**, recombinantly produced Fab fragment and monoclonal antibodies described above.

L32 ANSWER 27 OF 263 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AN 2000:277591 BIOSIS
TI Mercury binding polypeptides and nucleotides coding therefore.
AU Lopez, Osvaldo [Inventor, Reprint author]; Wylie, Dwane E. [Inventor]; Wagner, Fred W. [Inventor]
CS Walton, NE, USA ASSIGNEE: BioNebraska, Inc., Lincoln, NE, USA
PI US 5972656 October 26, 1999
SO Official Gazette of the United States Patent and Trademark Office Patents, (Oct. 26, 1999) Vol. 1227, No. 4. e-file.
AB **Metal binding** polypeptides which include an amino acid sequence coding for a variable region of a monoclonal antibody which immunoreacts with a mercury cation and nucleotides which include a nucleic acid sequence coding for the variable region are provided. The invention is also directed to **fusion proteins** which include a phage coat protein or portion thereof and the monoclonal antibody heavy chain variable region. The invention also provides bacteriophages which include the **fusion protein** in their coat. In addition, methods for detecting, removing, adding, or neutralizing mercuric cations in biological or inanimate systems through the use of the mercury binding polypeptides are provided.

L32 ANSWER 47 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 130:356743 CA
TI Whole cell- and protein-based biosensors for the **detection** of bioavailable heavy **metals** in environmental samples
AU Corbisier, Philippe; van der Lelie, Daniel; Borremans, Brigitte; Provoost, Ann; de Lorenzo, Victor; Brown, Nigel L.; Lloyd, Jonathan R.; Hobman, Jonathan L.; Csoregi, Elisabeth; Johansson, Gillis; Mattiasson, Bo
CS Vlaamse Instelling voor Technologisch Onderzoek (VITO), Boeretang 200, Mol, B-2400, Belg.
SO Analytica Chimica Acta (1999), 387(3), 235-244
AB The goal was to establish the feasibility of 2 biosensor technologies with enhanced specificity and selectivity for the **detection** of several bioavailable heavy **metals** in environmental samples. Two parallel strategies were followed. The 1st approach was to construct whole cell bacterial biosensors that emit a bioluminescent or fluorescent signal in the presence of a biol. available heavy **metal**. The mol. basis of σ-54 promoters as sensing elements of environmental pollutants was **detd.** and a no. of **metal**-induced promoter regions were identified, sequenced and cloned as promoter cassettes. The specificity of the promoter cassettes was detd. using luxCDABE reporter systems. Whole cell-biosensors contg. **metal**-induced lux reporter systems were incorporated into different matrixes for their later immobilization on optic fibers and characterized in terms of their sensitivity and storage **capacity**. The 2nd type of sensors was based on the direct interaction between **metal-binding** proteins and heavy **metal** ions. In this case, the **capacitance** changes of the proteins, such as synechococcal metallothionein (as a **GST-SmtA fusion protein**) and the **Hg** regulatory protein, **MerR**, were **detected** in the presence of fentomolar to millimolar **metal** ion concns.

L32 ANSWER 67 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 129:286801 CA
TI Detection of Heavy Metal Ions at Femtomolar Levels Using Protein-Based Biosensors
AU Bontidean, Ibolya; Berggren, Christine; Johansson, Gillis; Csoeregi, Elisabeth; **Mattiasson, Bo**; Lloyd, Jonathan R.; Jakeman, Kenneth J.; Brown, Nigel L.
CS Department of Biotechnology, Lund University, Lund, S-221 00, Swed.
SO Analytical Chemistry (1998), 70(19), 4162-4169
AB Sensors based on proteins (GST-SmtA and MerR) with distinct binding sites for heavy metal ions were developed and characterized. A capacitive signal transducer was used to measure the conformational change following binding. The proteins were overexpressed in Escherichia coli, purified, and immobilized in different ways to a self-assembled thiol layer on a gold **electrode** placed as the working **electrode** in a potentiostatic arrangement in a flow anal. system. The selectivity and the sensitivity of the 2 protein-based biosensors were measured and compared for copper, cadmium, mercury, and zinc ions. The GST-SmtA **electrodes** displayed a broader selectivity (sensing all 4 heavy metal ions) compared with the MerR-based ones, which showed an accentuated selectivity for mercury ions. Metal ions could be detected with both **electrode** types down to femtomolar concn. The upper measuring limits, presumably due to near satn. of the proteins' binding sites, were around 10-10M. Control **electrodes** similarly constructed but based on bovine serum albumin or urease did not yield any signals. The **electrodes** could be regenerated with EDTA and used for >2 wk with ~40% redn. in sensitivity.

L32 ANSWER 114 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 127:202246 CA

TI Reactions of complex metalloproteins studied by **protein**-film voltammetry
AU Armstrong, Fraser A.; Heering, Hendrik A.; Hirst, Judy
CS Inorganic Chemistry Laboratory, Oxford University, Oxford, OX1 3QR, UK
SO Chemical Society Reviews (1997), 26(3), 169-179
AB A review with many refs. The following review explores applications of voltammetric methods for observing reactions of complex metalloproteins. Attention is focused upon the technique of "**protein**-film voltammetry", in which the **protein** mols. under investigation are adsorbed on the **electrode** surface and electrochem. "interrogated.". The expts. address a minuscule sample with high sensitivity, and optimal control over both potential and time dependence of reactions. Factors governing the voltammetric response are outlined, and particular emphasis is given to the ability to study reactions that are coupled to and may "gate" the primary electron exchange processes. Examples described include proton-transfer and **metal-binding** reactions of iron-sulfur clusters, coupling of electron transfer in peroxidases, quantifying electron-transport pathways in multi-centered enzymes, and detection of "switches" that modulate the catalysis as a function of potential.

L32 ANSWER 148 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 123:280296 CA
TI Preparation and expression cloning of mercury-binding monoclonal antibody sequences and their use for immunoassays
IN Lopez, Osvaldo; Wylie, Dwane E.; Wagner, Fred W.
PA Bionebraska, Inc., USA
SO PCT Int. Appl., 107 pp.

PI	WO 9520607	A1	19950803	WO 1995-US1199 19950127
	EP 741749	A1	19961113	EP 1995-908736 19950127
	US 5972656	A	19991026	US 1997-888366 19970703
PRAI	US 1994-187407	A	19940127	

AB **Metal-binding** polypeptides and nucleic acids are provided which include an amino acid sequence coding for a variable region of a monoclonal antibody which immunoreacts with a mercury cation and a nucleotide sequence coding for the variable region. **Fusion proteins** and bacteriophages can include a phage coat protein or portion thereof and the monoclonal antibody heavy chain variable region. In addn., methods are described for detecting, removing, adding, or neutralizing Hg2+ in biol. or inanimate systems through the use of the mercury-binding polypeptides. Thus, hybridomas antibodies were produced with the spleen cells of BALB/c mouse that had received multiple injections of Hg2+ reacted with glutathione covalently bound to keyhole limpet hemocyanin. Seven hybridoma antibodies immunoreactive with glutathione-Hg2+ and specific for Hg2+ were cloned, PCR amplified, and the Fd and κ regions sequenced. ELISA assay utilizing BSA-glutathione added to polyvinyl chloride microtiter plates enabled detection of Hg2+ in a concn. as low as 10-9 M (0.2 ppb) with antibody IF10. Phagmid vectors are constructed to fuse the antibody Fd chain with the C-terminal domain of the coat protein cpIII of phage M13.

L32 ANSWER 172 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 121:273845 CA
TI Heterofunctional proteins and their use as affinity reagents
IN Kay, Brian K.; Fowlkes, Dana M.
PA University of North Carolina, USA
SO PCT Int. Appl., 256 pp.

PI	WO 9418318	A1	19940818	WO 1994-US977 19940201
	US 5498538	A	19960312	US 1993-176500 19931230
	US 5747334	A	19980505	US 1994-189331 19940131
PRAI	US 1993-13416	A	19930201	

AB A novel method for the prepn. of improved heterofunctional binding **fusion proteins** termed Totally Synthetic Affinity Reagents (TSARs) is described. These proteins are useful in diagnostics and therapeutics (no data). TSARs are concatenated heterofunctional proteins with at least two functional regions: a binding domain with affinity for a ligand and a second effector peptide portion that is chem. or biol. active and preferably connected via an optionally labile linker peptide. A library of chimeric genes encoding the biol. active peptide at one end of the gene with the other end made up of random oligonucleotides is screened by activity for constructs that bind the desired ligand. The construction and characterization of a no. of libraries in M13 using the pIII gene to ensure presentation of the random oligonucleotide-encoded domains and the screening of two of these libraries for ligands for a monoclonal antibody to a prostate carcinoma-specific antigen (mAb 7E11-C5) are demonstrated.

L32 ANSWER 199 OF 263 MEDLINE on STN
AN 93188698 MEDLINE
TI Isolation of a prokaryotic metallothionein locus and **analysis** of transcriptional control by trace **metal** ions.
AU Huckle J W; Morby A P; Turner J S; Robinson N J
CS Department of Biological Sciences, University of Durham, UK.
SO MOLECULAR MICROBIOLOGY, (1993 Jan) 7 (2) 177-87.
AB In eukaryotes, metallothioneins (MTs) are involved in cellular responses to elevated concentrations of certain **metal** ions. We report the isolation and analysis of a prokaryotic MT locus from Synechococcus PCC 7942. The MT locus (smt) includes **smtA**, which encodes a class II MT, and a divergently transcribed gene, smtB. The sites of transcription initiation of both genes have been mapped and features within the smt operator-promoter region identified. Elevated concentrations of the ionic species of **Cd**, Co, Cr, **Cu**, **Hg**, Ni, **Pb** and **Zn** elicited an increase in the abundance of **smtA** transcripts. There was no **detectable** effect of elevated **metal** (**Cd**) on **smtA** transcript stability. Sequences upstream of **smtA**, fused to a promoterless lacZ gene, conferred **metal**-dependent beta-galactosidase activity in Synechococcus PCC 7942 (strain R2-PIM8). At maximum permissive concentrations, **Zn** was the most potent elicitor in vivo, followed by **Cu** and **Cd** with slight induction by Co and Ni. The deduced SmtB polypeptide has similarity to the ArsR and CadC proteins involved in resistance to arsenate/arsenite/antimonite and to **Cd**, contains a predicted helix-turn-helix DNA-binding motif and is shown to be a repressor of transcription from the **smtA** operator-promoter.

L32 ANSWER 212 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 117:65075 CA
TI Cyanobacterial metallothionein gene expressed in Escherichia coli. Metal-binding properties of the expressed protein
AU **Shi, Jianguo**; Lindsay, William P.; Huckle, James W.; Morby, Andrew P.; Robinson, Nigel J.
CS Dep. Biol. Sci., Univ. Durham, Durham, DH1 3LE, UK
SO **FEBS** Letters (**1992**), 303(2-3), 159-63
AB The recently isolated Synechococcus gene smtA encodes the only characterized prokaryotic protein designated to be a metallothionein (MT). To examine the metal-binding properties of its product the smtA gene was expressed in Escherichia coli as a C-terminal extension of glutathione-S-transferase. The pH of half dissocn. of Zn, Cd, and Cu ions from the expressed protein was detd. to be 4.10, 3.50, 2.35, resp., indicating a high affinity for these ions (in particular for Zn in comparison to mammalian MT). E. coli expressing this gene showed enhanced (ca. 3-fold) accumulation of Zn.

L32 ANSWER 216 OF 263 CA COPYRIGHT 2003 ACS on STN

AN 116:52982 CA
TI **Metal-binding** peptides for purification of heterologous proteins by affinity chromatography
IN Sharma, Satish K.
PA Upjohn Co., USA
SO PCT Int. Appl., 39 pp.

PI	WO 9115589	A1	WO 1991-US1543	19910311
	US 5594115	A	US 1994-365994	19941229
PRAI	US 1990-506605	A2	19900409	

AB A method for purifying an heterologous protein manufd. in a recombinant host by immobilized metal affinity chromatog. (IMAC) is described. The method involves addn. of a sequence encoding a peptide contg. a no. of histidines in the general form (His-X)n where X is any of several amino acids, and a suitable proteinase cleavage site at the peptide-heterologous protein junction. The protein is purified by IMAC and the peptide is cleaved, optionally as a method of elution of the protein, with the proteinase. Analogs of the reverse transcriptase of human immunodeficiency virus with such peptides as N-terminal addns. were manufd. by expression of synthetic genes in Escherichia coli. The presence of these peptides on the protein did not affect reverse transcriptase activity and the peptides were removed by cleavage with renin. Chromatog. of the proteins on immobilized Ni resulted in the reverse transcriptase being strongly bound needing 100 mM imidazole to elute it after washing free of bacterial proteins.

L32 ANSWER 217 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 115:200369 CA
TI Method for producing heterofunctional **fusion protein** with specificity for ligand of choice
IN Fowlkes, Dana M.; Kay, Brian K.
PA USA
SO PCT Int. Appl., 79 pp.

PI	WO 9112328	A1	WO 1991-US1013	19910214
	US 5935823	A	US 1995-420945	19950411
PRAI	US 1990-480420	A	19900215	

AB A method for prepg. a heterofunctional **fusion protein** with desired ligand specificity comprises (1) inserting downstream from an ATG condon of a vector a 1st sequence encoding a family of binding domains designed to have a desired specificity, and a 2nd sequence encoding a biol. or chem. active effector domain; (2) transforming appropriate host cells with the vectors and culturing the transformants to produce the **fusion proteins**; and (3) screening the **fusion proteins** for those having the desired binding specificity and the biol. or chem. activity. The 1st nucleotide sequence is obtained by mutagenesis. Using this technique, a plasmid encoding a modified sequence from the variable domain of a monoclonal antibody to lysozyme fused, via a cleavable linker, to β-galactosidase was prepd. and the chimeric gene was expressed in Escherichia coli. The **fusion protein** was purified by p-aminophenyl-1-thio-β-D-galactopyranoside-Sepharose affinity chromatog. and the fractions with β-galactosidase activity were pooled. The purified **fusion protein** had appropriate binding specificity.

L32 ANSWER 228 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 116:101318 CA
TI Expression of the pea gene PsMTA in E. coli. **Metal-binding** properties of the expressed protein
AU Tommey, Andrew M.; **Shi, Jianguo**; Lindsay, William P.; Urwin, P. E.; Robinson, Nigel J.
CS Dep. Biol. Sci., Univ. Durham, Durham, DH1 3LE, UK
SO FEBS Letters (1991), 292(1-2), 48-52

AB The pea (Pisum sativum L.) gene PsMTA has an ORF encoding a predicted protein with sequence similarity to class I metallothioneins (MTs). To **examine** the **metal-binding** properties of the PsMTA protein, it has been expressed in E. coli as a carboxyterminal extension of glutathione-S-transferase (**GST**). **Metal** ions were assocd. with the expressed protein when purified from lysates of E. coli grown in **metal** supplemented media. The pH of half-dissocn. of **Zn**, **Cd**, and **Cu** ions from the recombinant **fusion protein** was detd. to be 5.35, 3.95 and 1.45 resp., compared with equiv. ests. of 4.50, 3.00 and 1.80 for equine renal MT.

L32 ANSWER 229 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 114:144002 CA
TI Proton, calcium, and magnesium binding by **peptides** containing γ-carboxyglutamic acid
AU Cabaniss, Steven E.; Pugh, Kathleen C.; Pedersen, Lee G.; Hiskey, Richard G.
CS Dep. Chem., Univ. North Carolina, Chapel Hill, NC, 27599-3290, USA
SO International Journal of Peptide & Protein Research (1991), 37(1), 33-8
AB γ-Carboxyglutamic acid (Gla) is believed to bind Ca(II) ions and Mg(II) ions in prothrombin and other coagulation **proteins**. Binding consts. for H+, Ca(II) ions, and Mg(II) ions to Gla-contg. **peptides** are detd. using pH and ion selective **electrode** titrns. The binding consts. for **peptides** contg. a single Gla residue are similar to the consts. for malonic acid. **Peptides** contg. two Gla residues in sequence (di-Gla **peptides**) bind Ca(II) ions and Mg(II) ions more strongly. KMgL for the di-Gla **peptides** is similar to the site-binding const. for Ca(II) ions in denatured BF1. These di-Gla **peptides** may be useful analogs for **metal binding** by the disordered Gla domain in BF1.

L32 ANSWER 232 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 112:212122 CA
TI DNA distortion accompanies transcriptional activation by the metal-responsive gene-regulatory protein MerR
AU **Frantz, Betsy**; O'Halloran, Thomas V.
CS Dep. Chem., Northwestern Univ., Evanston, IL, 60208, USA
SO **Biochemistry** (**1990**), 29(20), 4747-51
AB Transcriptional regulation of the bacterial Hg+ resistance operon (mer) in response to nanomolar concns. of Hg+ is achieved by the allosterically modulated transcriptional activator protein MerR. Hg+ modification of MerR activates transcription, facilitating the conversion of a RNA polymerase complex with the mer promoter from the closed conformation to the strand-sepd., transcriptionally competent open complex. An Hg-Mer-R-induced structural alteration at the center of the promoter was detected in the presence or absence of RNA polymerase by use of chem. nucleases sensitive to variations in DNA secondary structure. This hypersensitivity correlates directly with transcriptional activation, lending further support to a previous proposal that a protein-induced distortion in local DNA structure can be the key step in an allosterically modulated transcription activation mechanism.

L32 ANSWER 250 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 104:47811 CA
TI Studies on the binding of copper to dopamine β-monooxygenase and other **proteins** using the copper(2+) ion-selective **electrode**
AU Syvertsen, Christian; Gaustad, Rolf; Schroeder, Knut; Ljones, Torbjoern
CS Dep. Chem., Univ. Trondheim, Trondheim, N-7055, Norway
SO Journal of Inorganic Biochemistry (1986), 26(1), 63-76
AB The binding of Cu2+ to native and Cu-free dopamine β-monooxygenase was investigated by potentiometric titrns. with a Cu2+-selective **electrode**. Stoichiometric formation consts. were detd. from regression anal. of the

resulting titrn. curves. A stoichiometry of 4 high-affinity binding sites for Cu2+ (log Kf ~11) per enzyme tetramer, with more binding sites of lower affinity (log Kf ~5-7) was established. The data for binding of the 1st 4 Cu2+ to the enzyme tetramer indicate interactions in the binding. Bovine serum albumin, metal-free carbonic anhydrase, and ovotransferrin were also titrated with Cu2+, and the formation consts. of both high-affinity binding sites and other sites were detd. The stoichiometries of the 1 high-affinity binding site of Cu2+ for carbonic anhydrase (log Kf ~10-12) and 2 sites for ovotransferrin (log Kf ~11) agree with the reported **metal-binding** properties of these **proteins**. The no. of high-affinity binding sites for bovine serum albumin was pH dependent.

L32 ANSWER 251 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 105:37665 CA
TI The blue copper binding site: from the rack or tailor-made?
AU McMillin, David R.; Engeseth, Helen R.
CS Dep. Chem., Purdue Univ., West Lafayette, IN, 47907, USA
SO Biol. Inorg. Copper Chem., Proc. Conf. Copper Coord. Chem., 2nd (1986), Meeting Date 1984, Volume 1, 1-10. Editor(s): Karlin, Kenneth D.; Zubieta, Jon. Publisher: Adenine Press, Guilderland, N. Y.
AB A commonly held view is that the **protein** conformation dictates a strained geometry for Cu(II) in blue Cu **proteins** so as to foster high **electrode** potentials and facile electron transfer. Taking a somewhat different point of view, it is suggested here that the structure of the blue Cu site ought to be well adapted for binding Cu in order to promote the specific uptake of Cu. If this is true, the binding site of stellacyanin (I) should be similar to those of azurin (II) and plastocyanin (III), since the small blue **proteins** probably evolved from a common ancestor. Recent results were consistent with this expectation. The disulfide linkage of I was reassigned, and cysteine-87, which is homologous with the cysteine ligands of II and III, was available to bind Cu. 113Cd NMR studies confirmed the fact that the **metal-binding** sites of I and II were very similar, but they did not allow the identification of the 4th ligand of I unequivocally. There was, however, considerable indirect evidence implicating a disulfide donor which is, in the electronic sense, analogous to methionine S. Studies of the denaturation of native II, apo-II, and metal-substituted derivs. of II indicated that, compared with most other metal ions, Cu(II) binds quite strongly to the **protein**. The ligand field stabilization energy appeared to be an important factor in detg. the affinity for 1st row transition metal ions, and the deviation from tetrahedral symmetry appeared to bias the site toward Cu binding.

L32 ANSWER 260 OF 263 CA COPYRIGHT 2003 ACS on STN
AN 93:91423 CA
TI An **apoenzyme electrode**
AU **Mattiasson, Bo; Nilsson, Hans; Olsson, Bengt**
CS Chem. Cent., Univ. Lund, Lund, S-220 07, Swed.
SO Journal of Applied Biochemistry (1979), 1(5-6), 377-84
AB The use of immobilized **apoenzyme** for quantitation of the cofactor needed for enzymic activity is described. The sensitive part of an O **electrode** was covered by a nylon net onto which the **apoenzyme** was immobilized. In an alternative procedure a precolumn with immobilized **apoenzyme** was used. After exposure of the **apoenzyme electrode** to a cofactor-contg. sample, washing was carried out prior to administration of substrate. The **electrode** response obtained was proportional to the amt. of **apoenzyme** activated by the cofactor, resulting in a response proportional to the concn. of cofactor in the sample. In the system discussed here, Cu2+ was measured by the tyrosinase **apoenzyme**. By using this procedure, Cu2+ could be measured in

concns. below the micromolar level (50 ppb).

=> log y
STN INTERNATIONAL LOGOFF AT 13:23:25 ON 09 DEC 2003